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Action of the Unorganized Ferments of Malt Upon Carbohydrates.

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The Action of the Unorganized Ferments of Malt Upon Carbohydrates

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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INTRODUCTION

HISTORICAL.

The phenomena of digestion, that is, the decomposition of foods in such a manner as to change them into nutrient substances, is the basis of all life, yet very little of value was known until comparatively recent times. As early as the beginning of the sixteenth century, the process of digestion claimed considerable attention from many students, and the explanations set forth were many and different, the two principal theories being, one that digestion was due to mechanical work by the walls of the stomach, the other that it was due to the dissolving and transforming activity of the juices of the stomach. Réaumur (1) and Abbé Spallazani (1) performed very conclusive experiments confirming the latter theory. The differences of opinion concerning the phenomena of digestion greatly retarded the study of the enzymes, although the work of Réaumur and Abbé Spallazani did much to advance it, so it was not until the beginning of the eighteenth century, two centuries later, that the work along this line of investigation became prominent, and it was by the study of raw materials of the brewery, notably malt, that Dubrunfaut (1) laid the foundation of the study of enzymes. During all this time up to the beginning of the eighteenth century, the people of all countries had been manufacturing and consuming large quantities of wine, beer and other alcoholic drinks. Who was the first to make use of the act-

ive principles of the various cereals in order to bring about the desired results in the manufacture of the above commodities, we do not know, and it was not until the beginning of the eighteenth century that an attempt was made to explain what takes place when the reaction is made use of.

In 1812 Kirchoff (e) discovered that when he boiled starch with dilute H_2SO_4, a change took place, the starch disappearing as such and another substance being formed, which was proven to be a saccharine substance. In 1814, after a series of very interesting experiments, he proved that a similar reaction took place when he allowed malt to react with starch. In another experiment he noticed that fresh gluten can act under similar conditions on starch, dissolve it, and transform it into a saccharine substance. This experiment was taken up by Dubrunfaut (1), who in a long and masterly study, demonstrated that the activity of gluten is due to the presence of a small quantity of active substance originally in the raw grain, and that the quantity of this active substance is greatly increased when the grain is germinated. He proved finally, that the saccharine substance formed by the action of the enzyme of malt upon starch was not identical with the glucose which Kirchoff obtained by boiling starch with acid. Since then numerous experiments have been carried on in an endeavor to determine the exact nature of the substances formed when the enzymes of malt act upon starch, and many theories have been advanced. Pre-

vious to 1860, there was the belief that dextrin was isomeric with starch and midway between starch and dextrose, but in 1860 this idea was revolutionized when M. Musculus (h) came to the conclusion that diastase, as the enzyme of malt was called, had no action on dextrin, and that dextrin and grape sugar simultaneously appear when starch is acted upon by diastase, in the proportion of one part of grape sugar to two parts of dextrin. Since 1860 much work has been done concerning the kind of saccharine substances formed, gradually throwing more and more light upon the subject, until in 1895, when A. R. Ling (c) and Julian L. Baker came to the conclusion that a triose $C_{18}H_{32}O_{16}$ was the product of their reaction. In 1896, however, the same authors (d) had obtained sufficient data showing that in reality, the products of the reaction were a series of malto-dextrins, gradually decreasing in molecular weight and increasing in reducing power. Lately there have been no very radical changes in the theories concerning the substances formed when diastase acts upon starch, and the theory that is generally accepted is that maltose and dextrin are present and are formed simultaneously, in that the starch molecule, being very complex, breaks up into a molecule of maltose, $C_{12}H_{22}O_{11}$ and a primary dextrin of a complex molecular weight, and that this complex dextrin is acted upon by the diastase forming a second molecule of maltose and a secondary dextrin of a smaller molecular weight than the primary dextrin, and so on, the dextrins formed

gradually decreasing in molecular weight. Osborn (j) has recently done considerable work on the proteids of barley and in his investgations with barley malt, he has proven that the diastase of malt is made up of a series of proteid-like substances that coagulate at different temperatures.

GENERAL PROPERTIES OF ENZYMES.

In dealing with the subject of enzymes the question naturally arises, what are enzymes? This question is best answered by a definition, (1) stating that enzymes, soluble ferments or diastases, as they are called, are active organic substances secreted by cells, that have the property under certain conditions, of facilitating chemical reactions between certain bodies, without entering into the composition of the definite products which result. Enzymes are believed to be albuminoid substances, and consist usually of not only one substance, but of a series of closely related albuminoid substances. The enzymes are precipitated from aqueous solution by means of 80-90% alcohol, (n) but if allowed to remain in contact with the alcohol for some time, the lose most of their activity. The different enzymes differ in their solubility in water, some being very soluble, while others are difficultly soluble. Enzymes have the peculiar property in that they can be mechanically precipitated from solutions. (1) This has been demonstrated in several ways, a simple method being as follows: To a filtered and clear infusion of malt, a very dilute solution of sodium phosphate

is added, and then a solution of a calcium salt; there is produced in the liquid a precipitate of calcium phosphate, which settles gradually to the bottom of the vessel. A clear liquid is decanted, the precipitate filtered and washed, and the powder thus obtained possesses all the properties of a infusion of malt, being able to transform starch into maltose. By this method it is possible to obtain all of the enzymes in the solution. There is one condition necessary, however, and that is, that the substances used in the precipitation shall be harmless to the enzymes. Magnesium carbonate and aluminium hydrate may also be used in the process. Most of the enzymes, among them the enzymes of malt and yeast, are not very sensitive to the action of antiseptics. (i) The action of heat on the enzymes is very important. At 0° C. the diastatic action of the enzymes is so small as to be imperceptible. The action is intensified if the temperature is raised to 40° ; from 40° to 50° there is a marked increase; at 50° the maximum is supposed to be reached, (s) above which point the reaction diminishes; at 80° there is a cosiderable weakening produced and above 90° the diastases are wholly destroyed. (1) This statement is for diastases in general. There is some variation in the temperatures that are most favorable to the enzymes of malt, as will be seen from the experimental data covering this point, in connection with this work. T. B. Osborn found that the conversion of starch increases with the temperature until the heat reaches a point at which albu-

men begins to coagulate, and when the heat is sufficient to coagulate all the albumen, the reaction ceases. (j,k) In 1883, C. Lintner Jr. showed that in fifteen samples of malt, the diastatic power was very nearly proportional to the amount of coagulable albumen in the malt. (j) In the dry state diastases can stand a temperature of 90° and more, but without exception, they all lose their activity when their aqueous solutions are brought up to a temperature of 100°C. This fact relates enzymes in a striking manner to living organic matter. The exact chemical compositions of the enzymes are not known. The results of the various experiments are discordant, probably due to the fact that the enzymes are not in a pure state, but contain foreign substances. Below are some analyses of the diastases from malt. (1)

Carbon	Hydrogen	Nitrogen	Ash	Experimenter
45.67	6.90	4.57	6.08	Krauch
47.57	6,49	8.13	3.16	Zulkowski
46.66	7.35	10.41	4.79	Lintner

Some of the enzymes contain sulphur others do not. In some the nitrogen content is high, and would lead one to suppose that the enzyme was an albuminoid substance, and in others the nitrogen content is low. Diastases can diffuse through parchment membranes, and this distinguishes them from albuminoid substances. The general belief, however, is that the enzymes are very closely related to the albuminoid bodies. (j)

MANNER OF ACTION OF THE ENZYMES.

The chemical analysis of an enzyme is not sufficient to characterize it. To determine exactly the nature of an enzyme, the manner of action, the chemical change it can produce, and the substances upon which it acts must be observed. Enzymes can, according to their natures, produce very differen chemical reactions. Some of the enzymes act like oxidizing agents. An example of this kind of reaction is the transformation of hydrochinon into chinon:

$$C_6H_4\begin{matrix}OH\\OH\end{matrix} + O = C_6H_4\begin{matrix}O\\O\end{matrix} + H_2O \quad (1)$$

Another series of enzymes act on the molecule of substance and decompose it without causing oxidation or hydration. Of this class are the ferments of yeast, causing alcoholic fermentation according to the equation:

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_5OH. \quad (o)$$

A third series of enzymes, and the ones that were made use of in this work, is the series that cause hydrolysis, in that they cause one or more molecules of water to unite with the substances on which they act. These reactions are best shown by the following equations:

$$\underset{\text{(starch)}}{3(C_6H_{10}O_5)x} + H_2O = \underset{\text{(maltose)}}{C_{12}H_{22}O_{11}} - \underset{\text{(dextrin)}}{C_6H_{10}O_5} \quad (1)$$

$$\underset{\text{(cane-sugar)}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{(dextrose)}}{C_6H_{12}O_6} + \underset{\text{(laevulose)}}{C_6H_{12}O_6} \quad (o)$$

This decomposition of the molecules with hydration also takes place in the transformation of glucosides by enzymes. (o) When an infusion of malt is allowed to act on starch, the reaction takes place in two distinct stages. The first transformation taking place, is a liquefaction of the starch, the greater the diastatic power of the infusion , the more rapid is the liquefaction. This liquefied starch has an insipid taste, contains dextrins and only a trace of sugar. If this solution of dextrin s is cooled, some more infusion added, allowed to act at 50°-60°C., and samples taken from time to time analyzed, it is found that the dextrins gradually disappear, and there appears in the liquid a reducing sugar, maltose. The successive changes which occur under the action of the infusion of malt on the starch, may be easily watched by means of tincture of iodine. The deep blue color, obtained in the solution of starch by the iodine, gradually weakens as the saccharification proceeds, passing through violet, red, brown, yellow and disappearing entirely when the reaction is far advanced. The two diastatic functions, liquefaction and saccharification, have raised the question as to whether or not these two functions are due to two separate enzymes in the malt, but as yet there has been no separation of the enzymes, so that one possessed the liquefying power, and the other the saccharifying power. (1) When the diastase of malt, or amylase as it is called actd upon starch paste, the action is energetic, but when it acts upon the starch

granules, the granules are first corroded, then pass into solution, and are finally transformed into sugar. According to Petit, the ratio of maltose to the dextrine formed varies with the temperature employed in the saccharification. He found that as the temperature is raised, the ratio of the dextrin to the maltose increases from .4 to 1.00 at 60°-61° C., to 3 to 1 at 72-73 C. At the same time malto-dextrins of varying composition are formed, and here also the quantity depends largely on the temperature. At 60°, 2.4% were formed; at 65°, 6.6% were formed, and at 69°, 16.2% were formed. From the above it is evident that the quantities of the various substances depends largely on the temperature employed for saccharification. By employing a temperature below 50°, maltose and free dextrin are formed without malto-dextrins. By allowing the action to take place between 55° and 62°, the appearance of malto-dextrins is observed, and the percentage of the malto-dextrins increases rapidly with the rise in temperature. Above 70° the increase of dextrin in the malto-dextrin becomes evident.

DEVELOPMENT OF THE AMYLASE IN THE GRAIN.

As stated in the historical section of this work, there is present in the original grain only a very small quantity of the enzyme, and the quantity is greatly increased by germination.(p) All cereals produce amylase during germination, but barley furnishes the greatest yield of active substances. The developement of the enzyme in the grain is effected by means of a process called "malting". The process is more or less complicated. The first step is the sorting of the barley by means of mechanical separators. The sorted barley is then " steeped" as it is called, that is, put into large vats and covered withwater, the aim of the soaking being to make the grain absorb sufficient water for good germination; this can also be done by exposure to a blast of moist air. The grains swell and absorb a certain amount of oxygen, but at the same time the water dissolves out some of the soluble substances other than starch, the loss of extractive substances being about one per cent. (1) The water is frequently changed so that the dissolved substances cannot set up fermentation. The grain is allowed to soak from three to five days, and in that time the kernels absorb about fifty per cent of water. The water, which is frequently changed also serves to wash the grain. The grains are then transformed to the malting floors, made of cement, and spread evenly, so as to make a layer of grain about six inches high. The rooms in which the germination is allowed to take place, are kept at constant temperature.

Thus the grain is allowed to germinate, being turned frequently, so that the little roots are kept from tangling, and the grain is kept moist by sprinkling from time to time. When the plumules are about three- fourths of the length of the grain, the amylase formed has reached its maximum, but sometimes the maximum is reached before the plumules are of this length. The real point of maximum quantit of the enzyme can only be determined by analysis. The temperature is kept constant, and ranges from 12° to 17 C°, and the length of time required for the germination depends upon the method employed, usually requiring however, from four to seven days, and sometimes longer. When the amount of amylase has reached the desired point further germination is stopped by means of drying. The drying is done in a kiln by the aid of a coke fire, which is smokeless, and the temperature employed depends upon the flavor and color of the malt desired. The malt just before drying contains from ten to twelve per cent of water, and so it is dangerous to heat it above fifty degrees, before it is perfectly dehydrated, as the amylase changes under the action of heat, and this change is more rapid, the greater the amount of water present. During germination the grain secretes besides amylase, other active substances, among which are peptase, which transforms albuminoid substances into amides, and cytase, which acts upon certain kinds of cellulose. The role of the cytase is a very important one. The starch in the grain, is in the form of granules, enclosed within resistant cell-

walls. These cell-walls protect the starch against the action of the amylase, and the reaction would not be very strong, if the cytase did no liquefy the cell-walls of cellulose, enclosing the starch. After the malt has been kiln dried, it retains its activity for a long time, and it is impossible to germinate it again.(r)

OBJECT OF THE INVESTIGATION.

The object of this piece of work is to investigate some of the conditions that influence the action of the enzymes of malt upon the carbohydrates, as used industrially in the manufacture of beer, whiskey, alcohol and other kindred products. The points investigated are, the effect of temperature upon the reaction; the effect of time; the diastatic power; the effect of the different mineral salts in the proportions in which they are found in natural waters.

METHODS EMPLOYED.

The methods employed were practically the same as used in the brewing industry, except that they were carried out more carefully, greater attention being paid to details. The method of saccharification was as follows: A fraction of the normal weight, 26.048 grams, of the material was carefully weighed out and transferred to a 125 cc. Erlenmeyer flask. About 75 cc. of distilled water, or specially prepared water was added, the whole thoroughly mixed by shaking, the flask weighed properly and transferred to a thermostat, the temperature of which was kept constant at whatever temperature it was desired to work at. The flasks were allowed to remain in the thermostat for the desired length of time, after which they were removed and allowed to cool. The solution must be filtered from the residual matter and from the coagulated albumin. This was accomplished by means of Hirsch funnels and an asbestos film. Considerable difficulty was at first en-

countered in the filtration, but the use of the Hirsch funnels overcame all of them. The filtrate and washings are diluted to 200 cc. and the proper calculations made for the volume. In using the Schmidt and Haensch polariscope, the solution ought to be made up to 100cc., but this allowed only 25 cc. of water for washing and in consequence, all of the saccharine matter was not washed out of the residual matter, thus giving low results. By diluting to 200 cc. however, 125 cc. of water could be used for washing, and it was found that all of the saccharine matter was washed from the residual matter. Some of the solution was transferred to a 200 ~~cc.~~ mm. polariscope tube, and the amount of rotation determined by the polariscope.

THE POLARISCOPE.

The instrument used was a Schmidt and Haensch triple field polariscope. This instrument is graduated to read direct percentage of sucrose, if the normal weight, 26.048 grams of sugar is dissolved in water and made up to 100 cc. of solution. If the reading is taken in a 200 mm. tube, the reading will be 100 divisions of the scale. Therefore, if the normal weight of any substance be dissolved and made up to 100 cc. the reading of divisions on the polariscope scale will be the direct percentage of sucrose (q). But the saccharine matter formed in the saccharification of the malt is not sucrose, but maltose, dextrin and malto-dextrine, and these having more or less power than sucrose in de-

[illegible] in the [illegible]. It was [illegible] [illegible]

[illegible] all of them. The [illegible] and [illegible] were [illegible] to [illegible], and the proper [illegible] [illegible] for the [illegible]. [illegible] for the [illegible] and [illegible] [illegible]. The [illegible] [illegible] made up to [illegible], and [illegible] with 10 cc. of [illegible] [illegible] [illegible], all of the [illegible] [illegible] [illegible] [illegible] out of the residual [illegible], [illegible] [illegible] the [illegible] [illegible] in 500 cc. [illegible], [illegible] the [illegible] of [illegible] [illegible] [illegible] results, and it was found that all of the [illegible] [illegible] [illegible] from the residual [illegible]. Some of the [illegible] [illegible] in a 500 cc. [illegible], and the [illegible] of [illegible] [illegible] at the [illegible].

The [illegible].

The instrument used was a Babcock and Garrett triple [illegible] type. This [illegible] is [illegible] to [illegible] [illegible] of [illegible], at the normal [illegible], 20.00 [illegible] [illegible] in water and [illegible] to 100 cc. at [illegible]. [illegible] [illegible] in a 100 cc. tube, the reading will be [illegible] divisions of the scale. Therefore, if the normal weight of [illegible] [illegible] [illegible] [illegible] 100 cc., the reading of [illegible] [illegible] [illegible] would [illegible] [illegible] [illegible]. With the [illegible] [illegible] [illegible] [illegible] [illegible] of the scale [illegible] [illegible], [illegible] [illegible], [illegible] [illegible] of the [illegible] [illegible] [illegible]

flecting polarized light, we can readily see that the sum of their deflection might be more than 100 divisions on the polariscope scale, and this is the case in most of the experiments. The determination of the relative quantities of maltose, malto-dextrine, and dextrines formed is not a part of this work, so as to simplify matters, the percentage of sugar formed is in every case calculated to dextrose. This necessitated a graduation of the polariscope in terms of dextrose and it was found that under the conditions employed in the experiments, one division of the polariscope scale, while indicating one per cent of sucrose in the solution, was equivalent to 0.67 % of dextrose, the value which is used in all of the experiments.

EXPERIMENTAL.

INFLUENCE OF TIME UPON THE REACTION.

One tenth normal weight, or 2.6048 grams of malt is taken, 50cc of distilled water were added, the whole thoroughly mixed and placed in a thermostat at 61° - 62° C. At the end of 2,5 hours and four hours, respectively, samples were taken, filtered, diluted to 100cc., portions transferred to polariscope tubes and the reading taken.

Table I

Exp. No.	Material used	Wt. materials taken	distilled H_2O	Time	Temp.	Vol. diluted to	reading	% sugar as dextrose
2a	Malt	2.6048	50 cc	2.5 hrs.	61°-62°C.	100cc	8.5	56.95
2b	"	"	"	"	"	"	8.5	56.95
3a	"	"	"	4 hrs.	"	"	9.2	61.64
3b	"	"	"	"	"	"	9.2	61.64

From Table 1 we see that the time element has considerable influence on the amount of saccharine matter formed, a difference of 1.5 hours causing an increase of saccharine matter equivalent to seven divisions of the polariscope scale. At first it was intended to allow the experiments to run for eight hours, but this made it necessary to put off filtration until the next day, and during this interval of time, changes took place in the solutions. It was found that the best results were obtained if the experiment was allowed to run for four hours, this giving ample time for filtration and reading, besides this, a longer time could scarcely be employed, on account of the length of time required to heat up the large bulk of water in the thermostat to the temperature required, especially if this temperature was higher than 65°C. Under the best of conditions, it required from two to two and a half hours to heat the water to 70°C, during the cold weather, when the room temperature was low.

INFLUENCE OF TEMPERATURE.

The influence of temperature is best seen by referring to the following table, giving the mean values of the results of the different experiments. ~~TABLE I~~.

TABLE II.

Exp. No.	Material Used	wt. mat. taken	distilled H_2O	Time	Temp.	Vol. dil. to	reading	% sugar as dextrose
4	Malt	2.6048	70 cc.	4 hrs.	62° C	200	4.5	62.80
5	"	"	"	"	64°	100	9.8	65.66
7	"	"	"	"	66°	200	4.9	65.66
8	"	"	"	"	68°	"	4.9	65.66
9	"	"	"	"	70°	"	4.9	65.66
10	"	"	"	"	75°	"	4.9	65.66
11	"	"	"	"	75°+	"	4.8	64.32

From the above data in the table we see that there is an increase from 62° C. to 64° C. after which the results are constant, showing that the maximum is reached at 64° C. At 75°+ we notice a decrease, showing that the limit of maximum per cent of sugar is 75° C. At 64° there appeared a slight precipitate of albumin, which increased with the temperature, being decided at 66°, heavy at 70° and very heavy at 75°. To give a better idea as to the influence of temperature upon the reaction, the following table is added, in which rice, a substance rich in carbohydrates, was added to the malt and in such quantity that there was no possibility that all of it would be acted upon:

TABLE III.

Exp. No.	Materials used	wt. mat. taken	distilled H_2O	Time	Temp.	Vol dil. to	reading	% Sugar as dextrose.
18	Malt Rice	2.6048 2.6048	75cc	4 hrs.	64°C	200	8	53.60
13	"	"	"	"	64.5°	"	8.5	56.95
15	"	"	"	"	66.5°	"	11.0	73.70
19	"	"	"	"	71.°	"	12.8	85.76

From the above table we see that the degree of saccharification depends on the temperature employed. The saccharification for the above quantities of materials remained constant at 85.76% sugar as dextrose, this being the maximum that could be obtained. From the above table it is also seen that the most favorable temperature to work at is between 70° and 71°, it not being necesary to carry the temperature any higher if the maximum can be obtained at that temperature.

DIASTATIC POWER OF THE MALT.

By the diastatic power is meant the amount of saccharification that the malt can produce. The diastatic power of the malt was obtained directly by adding varying amounts of carbohydrates in the form of starch in rise, to the malt and saccharifying until a quantity was reached that gave the greatest yield of saccharine

matter from an economic standpoint. To begin with, in order to get a basis upon which to work, equal quantities of malt and rice were used. The results are seen in Table III, showing that the maximum is at 71°C and gives 85.76 % sugar as dextrose. Next, a series of experiments were run, using one half the amount of rice that was used in the experiments tabulated in Table III. The results are tabulated below.

TABLE IV.

Exp. No.	Materials used	wt. mat. used	distilled H_2O	Time	Temp.	Vol. dil to.	reading	% sugar as dextrose.
14	Malt Rice	2.6048 1.3024	75cc	4 hrs.	66.5°C.	200	8.2	71.85
21	"	"	"	"	71°C	"	9.4	83.89

In experiment 21, Table IV, we obtain the maximum extract at 71 and the result is 83.89 % sugar as dextrose. The quantities of materials used were two parts of malt to one of rice. In experiment 19, Table III we see that if we use two of malt to two of rice, we get a saccharine extract amounting to 85.76 % sugar as dextrose. We see that doubling the amount of rice gives us an increase of only 1.87 % of dextrose, so that the conclusion is reached that experiment 21 is nearer correct for the diastatic power than experiment 19, and that the difference in the readings is in all probability due to the amount of sugar present in the rice. In order to see how nearly correct the result of the experiment was, another series of experiments was run, varying the amounts rice added, only slightly. the results are interesting

and show a gradual increase until the results obtained in experiment 21 are reached. The results may be compared in the following table.

TABLE V.

Exp. No.	Materials used.	wt mat. taken	distilled H_2O	Time	Temp.	Vol. dil. to	reading	% sugar as dextrose
22	Malt Rice	2.6048 0.80	100 cc	4 hrs.	71°C	200	8.0	81.68
23	Malt Rice	2.6048 1.0000	100	"	"	"	8.5	82.23
24	Malt Rice	2.6048 1.200	100	"	"	"	9.1	83.40
21	Malt Rice	2.6048 1.3024	100	"	"	"	9.4	83.88
19	Malt Rice	2.6048 2.6048	100	"	"	"	12.8	85.76

From the above table we see that the amount of saccharine matter increases until the maximum is reached as in experiment 21, so the conclusion is drawn that for the above conditions, which are those most favorably carried out in the laboratory, the diastase of the malt can act upon the carbohydrates of the malt and also upon the carbohydrates of rice, equal to one-half the weight of the malt itself. Industrially, however, the amount of rice added to the malt is in considerably greater proportion, due to methods employed which cannot be duplicated in the laboratory, the modification consisting in changing the starch granules into starch paste by

boiling under pressure.

INFLUENCE OF MINERAL SALTS.

The character of the water used in brewing, whiskey manufacture, and kindred trades which make use of the conversion of starch into sugar by means of diastase, is of great importance, in that it directly affects the finished products, besides interfering more or less in the process of manufacture. It is known that certain substances have injurious effects upon the reaction, while others have no apparent effect whatever, so the present work was undertaken in order to see if the different mineral salts caused any variation in the saccharification. The salts used were chemically pure and solutions were made up of the different strengths that were likely to occur in natural waters. The range of strengths ran from 1 , through 3, 5, 10, 15, and 25 grains per U. S. gallon. The salts used were potassium chloride, potassium nitrate, potassium sulphate, sodium chloride, sodium nitrate, sodium sulphate, calcium chloride, calcium sulphate, calcium bicarbonate, magnesium sulphate, magnesium bicarbonate, and also city water. The magnesium and calcium bicarbonates were prepared by passing carbon dioxide gas into an aqueous solution of the normal carbonates until the precipitates dissolved and the solutions became distinctly acid. The solutions were filtered and made up to a known volume and the bicarbonates determined in aliquot parts. The results obtained can best be seen from the following tables giving the mean

values of the experiments tabulated in the last pages of the work.

Table VI.

Exp. No.	Materials used.	wt. mat. taken.	water containing.	am't H_2O	Time	Temp.	reading	% sugar as dextrose	decrease in % from normal (Exp. 21)
25	Malt- Rice	2.6048 1.3024	KCl - 1 grain per U.S. Gallon.	75cc.	4 hrs.	70°	9.3	82.99	0.89
26	"	"	3 grains per U.S. gallon	"	"	"	9.3	82.99	0.89
27	"	"	5 grains per U.S. gallon	"	"	"	9.2	82.10	1.78
28	"	"	10 grains per U.S. gallon	"	"	"	9.0	80.31	2.67

The effect of potassium chloride seems to increase with the quantity, and is almost proportional to the quantity present.

Table VII.

Exp. No.	Materials used.	wt. mat. taken.	water containing	am't H_2O	Time	Temp	reading	% sugar as dextrose.	decrease in % from normal.
29	Malt- Rice	2.6048 1.3024	KNO_3 - 1 grain per U.S. gallon	75cc	4 hrs.	70°	9.1	81.21	2.67
30	"	"	3 grains per U.S. gallon	"	"	"	9.0	80.31	3.56
31	"	"	5 grains per U.S. gallon	"	"	"	9.0	80.31	3.56
32	"	"	10 grains per U.S. gallon	"	"	"	9.0	80.31	3.56

One grain of potassium nitrate causes considerable decrease. Three grains causes a greater decrease still, beyond which there is no further decrease upon the addition of more potassium nitrate.

Table VIII.

Exp No.	Materials used	wt. mat. taken.	water containing	am't H_2O	Time	Temp.	reading	% sugar as dextrose.	decrease in % from normal
33	Malt- Rice	2.6048 1.3024	K_2SO_4 - 1 grain per U.S. gallon	75cc	4 hrs.	70°	9.3	82.99	0.89
34	"	"	3 grains per U.S. gallon	"	"	"	9.2	82.10	1.78
35	"	"	5 grains per U.S. gallon	"	"	"	9.2	82.10	1.78
36	"	"	10 grains per U.S. gallon.	"	"	"	9.2	82.10	1.78

Three grains of potassium sulphate seems to cause the maximum decrease, which however, is only half of the decrease caused by the same amount of potassium nitrate.

TABLE IX.

Exp No.	Materials used.	wt. mat. taken.	water containing	am't H_2O	Time	Temp	reading.	% sugar as dextrose	decrease in % from normal.
37	Malt Rice	2.6048 1.3024	NaCl - 1 grain per U.S. gallon.	75cc.	4 hrs.	70°	9.0	80.37	3.56
38	"	"	3 grains per U.S. gallon.	"	"	"	8.8	78.53	5.35
39	"	"	5 grains per U.S. gallon.	"	"	"	8.8	78.53	5.35
40	"	"	10 grains per U.S. gallon.	"	"	"	8.7	77.63	6.25

Sodium chloride seems to have a more injurious effect than any other salt used.

TABLE X.

Exp No.	Materials used.	wt. mat. taken.	water containing	am't H_2O	Time	Temp	reading.	% sugar as dextrose	decrease in % from normal.
44	Malt Rice	2.6048 1.3024	$NaNO_3$ - 1 grain per U.S. gallon	75cc	4 hrs.	70°	9.4	83.88	0.0
45	"	"	3 grains per U.S. gallon.	"	"	"	9.2	82.10	1.78
41	"	"	5 grains per U.S. gallon.	"	"	"	9.0	80.31	3.56
42	"	"	10 grains per U.S. gallon.	"	"	"	9.0	80.31	3.56

From Table X we see that I grain of sodium nitrate has no apparent effect, while 3 grains causes considerable decrease, while it is doubled if 5 grains are used. 10 grains causes no more decrease than 5 grains.

TABLE XI.

Exp. No.	Materials Used.	wt. mat. taken.	water containing.	am't H_2O	Time	Temp.	reading.	% sugar as dextrose.	decrease in % from normal.
46	Malt- Rice	2.6048 1.3024	Na_2SO_4 - 5 grain per U.S. gallon	75cc	4 hrs.	70°	9.4	83.88	0.0
47	"	"	10 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0
48	"	"	15 grains per U.S. gallon	"	"	"	9.4	83.88	0.0
49	"	"	25 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0

From the above table we see that sodium sulphate, even if used in quantities equal to 25 grains per gallon, has no effect whatever upon the reaction.

TABLE XII.

Exp. No.	Materials Used.	wt. mat. taken.	water containing.	am't H_2O	Time	Temp.	reading.	% sugar as dextrose	decrease in % from normal.
53	Malt- Rice	2.6048 1.3024	$CaSO_4$ - 5 grains per U.S. gallon.	75	4 hr.	70°	9.4	83.88	0.0
54	"	"	10 grains per U.S. gallon	"	"	"	9.4	83.88	0.0
55	"	"	15 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0
56	"	"	25 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0

Calcium sulphate has no apparent effect upon the reaction, as is shown by the above table. In fact, it is beneficial, since it is believed to have a desirable effect upon the yeast.

TABLE XIII.

Exp No.	Materials used.	wt. mat. taken.	water containing	am't H_2O	Time	Temp.	reading	% Sugar as dextrose	decrease in % from normal.
51a	{Malt {Rice	2.6048 1.3024	$CaCl_2$ - 5 grains per U.S. gallon.	75cc	4 hrs.	70°	9.0	80.32	3.56
51b	"	"	"	"	"	"	9.0	80.32	3.56
52a	"	"	10 grains per U.S. gallon.	"	"	"	9.0	80.32	3.56
52b	"	"	"	"	"	"	9.0	80.32	3.56

Calcium chloride has an injurious effect if 5 grains per gallon are present. 10 grains gave no further decrease.

TABLE XIV.

Exp. No.	Materials used.	wt. mat. taken.	water containing	am't H_2O	Time	Temp.	reading	% Sugar as dextrose.	decrease in % from normal.
57	{Malt- {Rice	2.6048 1.3024	$MgSO_4$ - 5 grains per U.S. gallon.	75cc.	4 hrs.	70°C.	9.4	83.88	0.0
58	"	"	10 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0
59	"	"	15 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0
60	"	"	25 grains per U.S. gallon.	"	"	"	9.4	83.88	0.0

From the above table we see that magnesium sulphate, like calcium sulphate has no effect whatever upon the reaction even in such large quantities as 25 grains per gallon.

TABLE XV.

Exp. No.	Materials used.	wt. mat. taken.	water containing.	om't H_2O	Time	Temp.	reading.	% Sugar as dextrose	decrease in % from normal.
61	{Malt {Rice	2.6048 1.3024	$CaH_2(CO_3)_2$ - 5 grains per U.S. gallon	75cc	4 hrs.	70°	9.0	80.32	3.56
62	"	"	10 grains per U.S. gallon	"	"	"	9.0	80.32	3.56
63	"	"	15 grains per U.S. gallon	"	"	"	9.0	80.32	3.56
64	"	"	25 grains per U.S. gallon.	"	"	"	9.0	80.32	3.56

Calcium bicarbonate has an injurious effect, as seen from the above table, the effect remaining constant no matter what the quantity added.

TABLE XVI.

Exp. No.	Materials used.	wt. mat. taken.	water containing.	am't H_2O	Time	Temp.	reading.	% Sugar as dextrose.	decrease in % from normal.
65	Malt- Rice	2.6048 1.3024	$MgH_2(CO_3)_2$ - 5 grains per U.S. gallon	75cc.	4 hr.	70°	9.0	80.32	3.56
66	"	"	10 grains per U.S. gallon.	"	"	"	9.0	80.32	3.56
67	"	"	15 grains per U.S. gallon.	"	"	"	9.0	80.32	3.56
68	"	"	25 grains per U.S. gallon.	"	"	"	9.0	80.32	3.56

From Table XVI we see that magnesium bicarbonate acts exactly like calcium bicarbonate, in that 5 grains per gallon gives a considerable decrease and this decrease is no greater when greater quantities of the salt are present.

TABLE XVII.

Exp. No.	Materials used.	wt. mat. taken.	water containing.	am't H_2O	Time	Temp.	reading.	% Sugar as dextrose.	decrease in % from normal.
50 a	Malt- Rice	2.6048 1.3024	From U. of I. wells. Tap water	75cc	4 hr.	70°	9.4	83.88	0.0
50 b	"	"	"	"	"	"	9.4	83.88	0.0

From the above table we see that water from the University Waterworks causes no decrease in the amount of saccharine matter produced, nevertheless its use is decidedly objectionable in that it imparts a deep reddish color to the solution. The following is an analysis of the water used: (from State Water Survey)

	Pts. per million	grains per gal.
NaCl	4.3	0.25
Na_2CO_3	74.7	4.36
$(NH_4)_2CO_3$	5.3	0.31

(Continued on next page.)

	Pts. per million	grs. per gal.
$MgCO_3$	108.1	6.3
$CaCO_3$	163.2	9.52
$FeCO_3$	3.5	0.2
Al_2O_3	1.2	.07
Silicious	18.6	1.08
Totals	378.9	22.09

From the above analyses we see that the water contains both calcium and magnesium bicarbonates, and from Table 17 we see that when this water is used in the experiment there is no decrease in the amount of saccharine matter formed. Now in tables 15 and 16 we see that both calcium and magnesium bicarbonates have a marked effect if five grains per U. S. gallon are present, while in the sample of University water, even larger quantities cause no perceptible decrease in the quantity of saccharine matter formed. The reasons for the above variations have not been accounted for, on account of lack of time, but the non-variation in the use of University tap water is probably due to the fact that the mineral salts contained in the water, tend to neutralize one another in such a manner that the water is as a whole, harmless in respect to the amount of saccharine matter formed, if used for saccharifying purposes.

From the above series of tables we see that mineral salts have an injurious effect upon the reaction, with the exception of

sodium sulphate, calcium sulphate and magnesium sulphate, which cause no change. The greatest change of all is observed when water containing 10 grains of sodium chloride is used, the results being almost double that of any other salt. It must also be remembered that the above results hold only for the sample of malt used in the experiment, and as there is considerable variation in different samples of malt used, the results obtained may vary somewhat . The results tabulated are the mean values for the different experiments, some of which were run in duplicate, some in triplicate, and some in quadruplicate. The sample of malt used may be considered an average sample of Al malt which gave the following analysis.

	I	II
Dry matter	94.62	94.66
Fat	2.62	2.63
Nitrogen as proteids	11.29	11.39
Ash	2.39	2.21
Dextrose	0.82	00.84
Sucrose	3.77	3.79
Starch	55.10	55.00
Crude Fiber(by difference)	18.63	18.80
	100.00	100.00

In conclusion may be added that the work was very interesting indeed, and there are many problems that may be studied to advantage, and about which very little is known, the chief one being as to what changes if any t ke place in the enzymes while the reaction is going on.

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Data

Exp. No.	Materials used	Wt. mat. taken	distilled H_2O	Time	Temp.	Vol. diluted to	reading on Polar.	% sugar as dextrose
1a	Malt	6.5120	50cc	8 hrs.	40°C	100cc	5	13.40
1b	"	"	"	"	"	"	5	13.40
2a	"	2.6048	60cc	2.5 hrs	61°-62°	"	8.5	56.95
2b	"	"	"	"	"	"	8.6	57.62
2c	"	"	"	"	"	"	8.5	56.95
3a	"	"	50cc	4 hrs.	"	"	9.3	62.31
3b	"	"	"	"	"	"	9.1	60.97
3c	"	"	"	"	"	"	9.2	61.64
3d	"	"	"	"	"	"	9.2	61.64
4a	"	"	"	"	62°	200	4.5	62.30
4b	"	"	"	"	"	200	4.5	62.30
4c	"	"	"	"	"	"	4.5	62.30
4d	"	"	"	"	"	"	4.5	62.30
5a	"	"	"	"	64°	100	9.8	65.66
5b	"	"	"	"	"	"	9.8	65.66
5c	"	"	"	"	"	"	9.8	65.66
5d	"	"	"	"	"	"	9.8	65.66
6a	"	"	70cc	"	"	200	4.9	65.66
6b	"	"	"	"	"	"	4.9	65.66
6c	"	"	"	"	"	"	4.9	65.66
6d	"	"	"	"	"	"	4.9	65.66
7a	"	"	"	"	66°	"	4.9	65.66
7b	"	"	"	"	"	"	4.9	65.66
7c	"	"	"	"	"	"	4.9	65.66
7d	"	"	"	"	"	"	4.9	65.66
8a	"	"	"	"	68°	"	4.9	65.66
8b	"	"	"	"	"	"	4.9	65.66
8c	"	"	"	"	"	"	4.9	65.66
8d	"	"	"	"	"	"	4.9	65.66

Exp. No.	Materials used.	Wt. mat. taken.	distilled H_2O	Time	Temp.	Vol. dil. to	reading	% sugar as dextrose.
9a	Malt	2.6048	70cc	4 hrs.	70°C.	200cc	4.9	65.66
9b	"	"	"	"	"	"	4.9	65.66
9c	"	"	"	"	"	"	4.9	65.66
9d	"	"	"	"	"	"	4.9	65.66
10a	"	"	"	"	75°c	"	4.9	65.66
10b	"	"	"	"	"	"	4.9	65.66
10c	"	"	"	"	"	"	4.9	65.66
10d	"	"	"	"	"	"	4.9	65.66
11a	"	"	"	"	75°+	"	4.8	64.32
11b	"	"	"	"	"	"	4.8	64.32
11c	"	"	"	"	"	"	4.8	64.32
12a	Malt Rice	2.6048 2.6048	75cc.	2.5 hrs.	64.5°	"	8.2	54.94
12b	"	"	"	"	"	"	8.2	54.94
13a	"	"	"	4 hrs.	"	"	8.5	56.95
13b	"	"	"	"	"	"	8.5	56.95
13c	"	"	"	"	"	"	8.5	56.95
14a	Malt Rice	2.6048 1.3024	75cc	"	66.5°	"	8.2	71.85
14b	"	"	"	"	"	"	8.2	71.85
14c	"	"	"	"	"	"	8.2	71.85
15a	Malt Rice	2.6048 2.6048	"	"	66.5°	"	10.9	73.03
15b	"	"	"	"	"	"	11.0	73.70
15c	"	"	"	"	"	"	11.0	73.70
16a	"	"	"	"	"	"	11.0	73.70
16b	"	"	"	"	"	"	11.0	73.70
16c	"	"	"	"	"	"	11.0	73.70

Exp No.	Materials used.	wt mat. taken.	distilled H_2O	Time.	Temp.	Vol dil. to.	reading.	% sugar as dextrose.
17a	Malt ~~ordinary starch~~	2.6048 2.6048	75 cc.	4 hrs.	66.5°	200	10	67.00
17b	"	"	"	"	"	"	10	67.00
17c	"	"	"	"	"	"	10	67.00
18a	Malt Rice	"	"	"	64°	"	8	53.60
18b	"	"	"	"	"	"	8	53.60
19a	"	"	"	"	71°	"	12.8	85.76
19b	"	"	"	"	"	"	12.8	85.76
19c	"	"	"	"	"	"	12.8	85.76
20a	Malt Rice	2.6048 3.9072	"	"	"	"	16.8	96.48
20b	"	"	"	"	"	"	16.8	96.48
20c	"	"	"	"	"	"	16.8	96.48
21a	Malt Rice	2.6048 1.3024	"	"	"	"	9.5	83.89
21b	"	"	"	"	"	"	9.5	83.89
21c	"	"	"	"	"	"	9.5	84.78
22a	Malt Rice	2.6048 0.800	"	"	"	"	8.0	81.68
22b	"	"	"	"	"	"	8.0	81.68
23a	Malt Rice	2.6048 1.0000	"	"	"	"	8.5	82.23
23b	"	"	"	"	"	"	8.5	82.23
24a	Malt Rice	2.6048 1.200	"	"	"	"	9.1	83.40
24b	"	"	"	"	"	"	9.1	83.40
~~25a~~	~~Malt~~							
~~25b~~								

Exp. No.	Materials used	wt. mat. taken	Water containing	am't H_2O	Time	Temp.	Vol. dil. to	reading	% dextrose
25a	Malt Rice	2.6048 1.3024	KCl - 1 grain per U.S. gallon	75cc.	4 hrs.	70°C.	200	9.3	82.99
25b	"	"	"	"	"	"	"	9.3	82.99
26a	"	"	3 grains per U.S. gallon.	"	"	"	"	9.3	82.99
26b	"	"	"	"	"	"	"	9.3	82.99
27a	"	"	5 grains per U.S. gallon.	"	"	"	"	9.2	82.10
27b	"	"	"	"	"	"	"	9.2	82.10
28a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.0	80.31
28b	"	"	"	"	"	"	"	9.0	80.31
29a	Malt Rice	2.6048 1.3024	KNO_3 - 1 grain per U.S. gallon	75cc	4 hrs.	70°C.	200	9.1	81.21
29b	"	"	"	"	"	"	"	9.1	81.21
30a	"	"	3 grains per U.S. gallon	"	"	"	"	9.0	80.32
30b	"	"	"	"	"	"	"	9.0	80.32
31a	"	"	5 grains per U.S. Gallon.	"	"	"	"	9.0	80.32
31b	"	"	"	"	"	"	"	9.0	80.32
32a	"	"	10 grains per U.S. Gallon.	"	"	"	"	9.0	80.32
32b	"	"	"	"	"	"	"	9.0	80.32
33a	Malt Rice	2.6048 1.3024	K_2SO_4 - 1 grain per U.S. gallon	75cc	4 hrs.	70°	200	9.3	82.99
33b	"	"	"	"	"	"	"	9.3	82.99
34a	"	"	3 grains per U.S. Gallon.	"	"	"	"	9.2	82.10
34b	"	"	"	"	"	"	"	9.2	82.10
35a	"	"	5 grains per U.S. gallon.	"	"	"	"	9.2	82.10
35b	"	"	"	"	"	"	"	9.2	82.10
36a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.2	82.10
36b	"	"	"	"	"	"	"	9.2	82.10

Exp. No.	Materials used.	Wt. mat. taken.	Water containing	am't H_2O	Time	Temp	Vol. dil to.	reading	% dextrose
37a	Malt- Rice	2.6048 1.3024	NaCl - 1 grain per U.S. Gallon.	75cc	4 hrs.	70°C	200	9.0	80.3✓
37b	"	"	"	"	"	"	"	9.0	80.3✓
38a	"	"	3 grains per U.S. gallon.	"	"	"	-	8.8	78.53
38b	"	"	"	"	"	"	"	8.8	78.53
39a	"	"	5 grains per U.S. gallon.	"	"	"	"	8.8	78.53
39b	"	"	"	"	"	"	"	8.8	78.53
40a	"	"	10 grains per U.S. gallon.	"	"	"	"	8.7	77.63
40b	"	"	"	"	"	"	"	8.7	77.63
41a	Malt- Rice	2.6048 1.3024	$NaNO_3$ - 5 grains per U.S. gallon	75cc	4 hrs.	70°C	200	9.0	80.3✓
41b	"	"	"	"	"	"	"	9.0	80.3✓
42a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.0	80.3✓
42b	"	"	"	"	"	"	"	9.0	80.3✓
44a	"	"	1 grain per U.S. gallon	"	"	"	"	9.4	83.88
44b	"	"	"	"	"	"	"	9.4	83.88
45a	"	"	3 grains per U.S. gallon.	"	"	"	"	9.2	82.10
45b	"	"	"	"	"	"	"	9.2	82.10
46a	Malt- Rice	2.6048 1.3024	Na_2SO_4 - 5 grains per U.S. gallon.	75cc	4 hrs.	70°C	200	9.4	83.88
46b	"	"	"	"	"	"	"	9.4	83.88
47a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.4	83.88
47b	"	"	"	"	"	"	"	9.4	83.88
48a	"	"	15 grains per U.S. gallon.	"	"	"	"	9.4	83.88
48b	"	"	"	"	"	"	"	9.4	83.88
49a	"	"	25 grains per U.S. gallon.	"	"	"	"	9.4	83.88
49b	"	"	"	"	"	"	"	9.4	83.88

Exp. No.	Materials used.	wt. mat. taken.	water containing	am't H_2O	Time	Temp.	Vol. dil. to.	reading	% dextrose
50 a	Malt Rice	2.6048 1.3024	Vol. I tap. H_2O. For analysis see pages 26 & 27.	75cc	4 hrs.	70°C	200	9.4	83.88
50b	"	"	"	"	"	"	"	9.4	83.88
51a	Malt Rice	2.6048 1.3024	$CaCl_2$ - 5 grains per U.S. gallon.	75cc	4 hrs.	70°C	200	9.0	80.3
51b	"	"	"	"	"	"	"	9.0	80.3
52a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.0	80.3
52b	"	"	"	"	"	"	"	9.0	80.3
53a	Malt Rice	2.6048 1.3024	$CaSO_4$ - 5 grains per U.S. gallon.	75cc	4 hrs.	70°C	200	9.4	83.88
53b	"	"	"	"	"	"	"	9.4	83.88
54a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.4	83.88
54b	"	"	"	"	"	"	"	9.4	83.88
55a	"	"	15 grains per U.S. gallon.	"	"	"	"	9.4	83.88
55b	"	"	"	"	"	"	"	9.4	83.88
56a	"	"	25 grains per U.S. gallon.	"	"	"	"	9.4	83.88
56b	"	"	"	"	"	"	"	9.4	83.88
57a	Malt Rice	2.6048 1.3024	$MgSO_4$ - 5 grains per U.S. gallon	75cc	4 hrs.	70°C.	200	9.4	83.88
57b	"	"	"	"	"	"	"	9.4	83.88
58a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.4	83.88
58b	"	"	"	"	"	"	"	9.4	83.88
59a	"	"	15 grains per U.S. gallon.	"	"	"	"	9.4	83.88
59b	"	"	"	"	"	"	"	9.4	83.88
60a	"	"	25 grains per U.S. gallon.	"	"	"	"	9.4	83.88
60b	"	"	"	"	"	"	"	9.4	83.88

Exp. No.	Materials Used	Mat. taken wt.	water containing	am't H_2O	Time	Temp.	Vol dil. to	reading	% dextrose
61a	Malt Rice	2.6048 1.3024	$CaH_2(CO_3)_2$ — 5 grains per U.S. gallon	75cc.	4hrs.	70°C.	200	9.0	80.3 ✓
61b	"	"	"	"	"	"	"	9.0	80.3 ✓
62a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.0	80.3 ✓
62b	"	"	"	"	"	"	"	9.0	80.3 ✓
63a	"	"	15 grains per U.S. gallon	"	"	"	"	9.0	80.3 ✓
63b	"	"	"	"	"	"	"	9.0	80.3 ✓
64a	"	"	25 grains per U.S. gallon.	"	"	"	"	9.0	80.3 ✓
64b	"	"	"	"	"	"	"	9.0	80.3 ✓
65a	Malt Rice	2.6048 1.3024	$MgH_2(CO_3)_2$ — 5 grains per U.S. gallon.	75cc	4hrs.	70°C.	200	9.0	80.3 ✓
65b	"	"	"	"	"	"	"	9.0	80.3 ✓
66a	"	"	10 grains per U.S. gallon.	"	"	"	"	9.0	80.3 ✓
66b	"	"	"	"	"	"	"	9.0	80.3 ✓
67a	"	"	15 grains per U.S. gallon.	"	"	"	"	9.0	80.3 ✓
67b	"	"	"	"	"	"	"	9.0	80.3 ✓
68a	"	"	25 grains per U.S. gallon.	"	"	"	"	9.0	80.3 ✓
68b	"	"	"	"	"	"	"	9.0	80.3 ✓